

**ntagonists**

rgos PGYRYLFA<sup>—</sup>CSP-LTRLRCQKQPC<sup>—</sup>KLFTTVKRQEF<sup>—</sup>LDENVNINSLCQC<sup>—</sup>PK---GHRCPSSHHTQSG- D. melanogaster

**gonists**

in-3 IEKLKEAKCKD-----YCHHNATCHVEVIFRED---RVSAVVPSC<sup>—</sup>HPQGWEGTRC<sup>—</sup>DRHYVQAF- C. elegans

ein DRASGIPCN--FD--YCFHNGTCRMI<sup>—</sup>PDIN-----EYI--CRCPTEYF<sup>—</sup>GNRCENKWPDSR- D. melanogaster  
 arken ETEIQMLPCSEAYNTSF<sup>—</sup>CLNGGHC<sup>—</sup>FQHPMVNN-----TVFHS<sup>—</sup>CLCVNDYDGER<sup>—</sup>CAYKSWNGD- D. melanogaster  
 pitz NITFPTYKCPETFD<sup>—</sup>AWYCLNDAH<sup>—</sup>CF<sup>—</sup>AVKIADL-----PVYS-CECAIGFM<sup>—</sup>QRC<sup>—</sup>EYKEIDNT- D. melanogaster  
 eren NVTFPIFA<sup>—</sup>CPPTYVAWYCLNDGT<sup>—</sup>CTVKIHNE-----ILYN-CECALGFM<sup>—</sup>GPRCEYKEIDGS- D. melanogaster

**RG1\_alpha**

TGTSHLVKCAEKEKT-FCVNGGEC<sup>—</sup>FMVKDLS-----NPSRYLCKCQ<sup>—</sup>PGFTGARCTEN<sup>—</sup>VPMKV- Homo sapiens

**RG1\_beta**

TGTSHLVKCAEKEKT-FCVNGGEC<sup>—</sup>FMVKDLS-----NPSRYLCKCPNE<sup>—</sup>FTGDR<sup>—</sup>CQNYVMAS<sup>—</sup>F- Homo sapiens

**RG2\_alpha**

SWSGHARKCNETAKS-YCVNGGV<sup>—</sup>CYYIEGIN-----QLS---CKCPNGFF<sup>—</sup>QRCLEK<sup>—</sup>LPLRL- Homo sapiens

**RG2\_beta**

SWSGHARKCNETAKS-YCVNGGV<sup>—</sup>CYYIEGIN-----QLS---CKCPVGYT<sup>—</sup>GDR<sup>—</sup>CQQFAMVNF- Homo sapiens

**RG3**

ERSEHF<sup>—</sup>KPC<sup>—</sup>RD<sup>—</sup>KDLA-YCLNDGEC<sup>—</sup>FVIE<sup>—</sup>TLTG-----SHK--HCRCKEGY<sup>—</sup>QGVRC<sup>—</sup>DQ-FLPKTD Homo sapiens

**RG4**

MPTDHEEP<sup>—</sup>CGPSHKS-FCLNGGL<sup>—</sup>CYVIPTIP-----SP---FCRCVENYT<sup>—</sup>GARCEE<sup>—</sup>VFLPGS- Homo sapiens

**3F**

SVRNSDSE<sup>—</sup>CLSHDG-YCLHDGV<sup>—</sup>CMYIEALD-----KYA---CNCVVG<sup>—</sup>YIGERCQYRD<sup>—</sup>LKWW- Homo sapiens

**3F\_alpha**

AVVSHFND<sup>—</sup>CPDSHTQ-FCFH-GT<sup>—</sup>CRFLVQED-----KPA---CVCHSGYV<sup>—</sup>GARCEHAD<sup>—</sup>LLAV- Homo sapiens

**stacellulin**

KRKGHFSR<sup>—</sup>CPKQYKH-YCIK-GR<sup>—</sup>CRFVVAEQ-----TPS---CVDEGYI<sup>—</sup>GARCERVD<sup>—</sup>LFYL- Homo sapiens

**aphiregulin**

RNRKKNP<sup>—</sup>CNAEFQN-FCIH-GE<sup>—</sup>CKYIEHLE-----AVT---CKCQ<sup>—</sup>QYFGER<sup>—</sup>CGEKSMKTH- Homo sapiens

**3-EGF**

GLGKKRDP<sup>—</sup>CL<sup>—</sup>RYKD-FCIH-GE<sup>—</sup>CKYV<sup>—</sup>KELR-----APS---CI<sup>—</sup>CHPGYHGER<sup>—</sup>CHGLSLPVE- Homo sapiens

**piregulin**

VAQVSITK<sup>—</sup>CS<sup>—</sup>SDMNG-YCLH-GQ<sup>—</sup>CIYLV<sup>—</sup>DMS-----QNY---CRCEVGYT<sup>—</sup>GVRCEH<sup>—</sup>FFLTVH- Homo sapiens

**igen**

VALKFSHP<sup>—</sup>CLEDHNS-YCIN-GA<sup>—</sup>CAFHH<sup>—</sup>ELK-----QAI---CR<sup>—</sup>CTGYT<sup>—</sup>GQRC<sup>—</sup>EHLLT<sup>—</sup>TSY- Mus musculus

nserved cysteine #: 1 2 3 4 5 6

**FIGURE 1**

# Replacement Sheet

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Dros._melanogaster -----MPTTLMMLPCMLLLLLTAAAVAVGGTRLPLEVFEITPTTS--TADKHKSL
Dros._virilis      MASIRAHSLLLLLLRLMLLPLPLLLLLLMLTGGAQSTRPLEVYELTPASADSDAKHKSL
Musca_domestica    -----MLSLTIFMLATHIINACYSTRLPLEVYELTPNAAGGTDLKHKNL
                   :*.  :::*      .  .*****:*.***:: :  ***.*

Dros._melanogaster QYTVVYDAK-----DISG-----AAAATG-----VASSTVKPATEQLTVVSISSTAA
Dros._virilis      EY-AIYDPK-----ELTGAPKAAAAAAATT-----TTSSTARPSSEKPLAIAVVSISAE
Musca_domestica    EYSTINGGSGQHFLAINGRSKQQHVSAAAMEEPKMLSSHDSKAAATKTLTVSSMGTPSA
                   :*  .:  . .      :.*      **:      :*      :.:  :  .:  . .

Dros._melanogaster EKDLAES-----RRHARQMLQKQQQ-----HRSIIGG---K
Dros._virilis      QQQQQQSELEPATQ-----AGRRARQMLQQQHR-----LSSSSSSSSNK
Musca_domestica    AATTTTSTSTATATATTNQLDRRRSRQMLDIMQKNHHDQTGNHKLPPVLSSGGAATG
                   *                      *:****:  ::                      *

Dros._melanogaster HGD--RDVRILYQVGDSEEDLPVCAPNAVCSKIDLYETPWIERQCRCQPESNRMPNNVIIH
Dros._virilis      HAHSV KDLRLILYQVGDSEADLPVCAPNAVCSKIDLYETPWIERQCRCQPESNRMPNNVIVH
Musca_domestica    ASHSQKDVRLILYQVGNSEDDLEPCAPNAVCSKIDLYETPWIERQCRCQPAVNRSPETIIH
                   ..  :*:*****:*  ***:*****  ** *:  :*  *
                   <----->
                   A1 domain

Dros._melanogaster HHSHSSGSVDS--LKYRNYEREKMMQHQR---MLLGEF---QDKKFESLHMKKLMQKLG
Dros._virilis      HHEHPHGTMSG--QKYRSYYEKEKLLQHQR---LLL-----DKKYESLHLKKLMQKLG
Musca_domestica    HHKETASHSNHNSEKYHTFYEHSKLAHQQQNKHLLDAA SFVGDKKFDNLHLKKLMHKLQ
                   **...  .      **::*:*:*:  :.:  :  :  :  :  :  :  :  :  :  :  :  :
                   :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :

Dros._melanogaster AVYEDDL-----DHLDQS-----PDYNDALP--YAEVQDNEFP-----RGSAHM
Dros._virilis      AVYEDDLQLPSAGDYVERS-----PDYNEALPPAYEELADNELPQ-----APARSATHM
Musca_domestica    AVYEDDLNLPSDYHRHEETNSALDDSEATLYYADEIKDNEFPAHFAMKROHLYSNTPHM
                   *****      .  :.:      *  *:      *  :  :  :  :  :  :  :  :  :  :
                   :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :

Dros._melanogaster RHSGHRG-SKEPATTFIGGCPSLGVEDGHTIADKTRHYKMCQPVHKLFPVCTHFRDYTWT
Dros._virilis      RHSGHRG-LKE-AVSFIGGCPSNLGVEDGHTIADKTRHYKLCQPVHKLFPVCKHFRDYTWT
Musca_domestica    RHSGHTGGGGGCKISYIGGCPSGLGIEDGHTIADKTRHYKMCQPVHKLFPVCRHFRDYTWT
                   ***** *  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :
                   <----->
                   A2 domain

Dros._melanogaster LTTAAELNVTEQIVHCRCPKNSVTYLTAKREPVPNSSTAYRYLFACSPLTRLRQQRKQPCPK
Dros._virilis      LTTAAELNVTEQVVHCRCPKNSVTYLTAKREPVPNSSTAYRYLFACSPLTRLRQQRKQPCPK
Musca_domestica    LTTSPENMTTEQIVHCRCPKNSVTYLTAKREPSEDGNGGYKYLFA CSPLTRFRQQRKQPCPK
                   ***:*.*:*.***:*****:*****:***  :..  .*:*****:*****
                   <----->
                   A2 (continued)                                     EGF domain

Dros._melanogaster LFTVRKRQEFLEVNINSLCQCPKGHRCPSHHTQSGVIAGESFLEDNIQTYSGYCMAND
Dros._virilis      LFTVRKRQEFLEVNINSLCQCPKGHRCPSHHTQSGVIAGESFLEDNIQTYSGYCMAND
Musca_domestica    LFTVRKRQEFIDEVNINALCQCPKGHRCPSHHTQSGVIAGETFLEDNIQTYSGYCMVND
                   *****:*****:*****:*****:*****:*****:*****:*****
                   <----->
                   EGF domain (continued)

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FIGURE 2

### FIGURE 3

HUMAN	Exon A	Exon B	Sequence ID #
	<-----	----->	----->
NRG1_alpha	...TGTSHLVKCAEKEKTF	CVNGGECFMVKDLSNPSRYLCKQPGFTGARTENVPMKVQ...	14
NRG1_beta	...TGTSHLVKCAEKEKTF	CVNGGECFMVKDLSNPSRYLCKPNEFTGDRCCQNYVMASFY...	15
Translated genomic locus	...TGTSHLVKCAEKEKTF	CVNGGECFMVKDLSNPSRYLCK*	73, 128
NRG2_alpha	...SWSGHARKCNETAKSY	CVNGGVYYIEGINQLS---CKPNGFFGQRCLEKLPRLY...	16
NRG2_beta	...SWSGHARKCNETAKSY	CVNGGVYYIEGINQLS---CKCPVGTGDRCCQFAMVNFY...	17
Translated genomic locus	...SWSGHARKCNETAKSY	CVNGGVYYIEGINQLS---CK*	74, 129
NRG3	...ERSEHFKPCRDKDLAY	CLNDGECFVIETLTGSH-KHCRCKEGYQGVRCDDQ-FLPKTD...	18
Translated genomic locus	...ERSEHFKPCRDKDLAY	CLNDGECFVIETLTGSH-KHCR*	75, 130
NRG4	MPTDHEEP	CGPSHKSFCLNGGLCYVIPTIPSPF---CRCVENYTGARCEEVFLPGSS...	19
Translated genomic locus	MPTDHEEP	CGPSHKSFCLNGGLCYVIPTIPSPF---CR*	76, 131
EGF	...SVRNSDSECP	LSHDGYCLHDGVCMYIEALDKYA---CNCVVGYIGERCQYRDLKWWE...	20
Translated genomic locus	...SVRNSDSECP	LSHDGYCLHDGVCMYIEALDKYA---CK*	77, 132
TGF_alpha	...AVVSHFND	CPDSHTQFCFH-GTCRFLVQEDKPA---CVCHSGVVGARCEHADLLAVV...	21
Translated genomic locus	...AVVSHFND	CPDSHTQFCFH-GTCRFLVQEDKPA---CV*	78, 133
Betacellulin	...KRKGHFSRCPKQ	KHYCIK-GRCRFVVAEQTPS---CVDEGYIGARCERVDLFYLR...	22
Translated genomic locus	...KRKGHFSRCPKQ	KHYCIK-GRCRFVVAEQTPS---CV*	79, 134

FIGURE 4A

Amphiregulin	...	PNRKKKNPCNAEFQNF <sup>-</sup> CIH-GE <sup>-</sup> CKYIEHLEAVT---CKQQ <sup>-</sup> EYFG <sup>-</sup> ERC <sup>-</sup> G <sup>-</sup> EKSMKTHS....	23
Translated genomic locus	...	PNRKKKNPCNAEFQNF <sup>-</sup> CIH-GE <sup>-</sup> CKYIEHLEAVT---CK*	80, 135
HB-EGF	...	GLGKKRDP <sup>-</sup> CLRK <sup>-</sup> YKDF <sup>-</sup> CIH-GE <sup>-</sup> CKYVVKELRAPS---CICH <sup>-</sup> PGYHGER <sup>-</sup> CHGLSLPVEN....	24
Translated genomic locus	...	GLGKKRDP <sup>-</sup> CLRK <sup>-</sup> YKDF <sup>-</sup> CIH-GE <sup>-</sup> CKYVVKELRAPS---CM*	81, 136
Epiregulin	...	VAQVSITK <sup>-</sup> CS <sup>-</sup> SDMNGY <sup>-</sup> CLH-GQ <sup>-</sup> CIYLVDMSQNY---CRCE <sup>-</sup> VGYTG <sup>-</sup> VRCEHFFLT <sup>-</sup> VHQ....	25
Translated genomic locus	...	VAQVSITK <sup>-</sup> CS <sup>-</sup> SDMNGY <sup>-</sup> CLH-GQ <sup>-</sup> CIYLVDMSQNY---CR*	82, 137
Epigen (Mouse)	...	VALKFSHP <sup>-</sup> CL <sup>-</sup> EDHNSY <sup>-</sup> CIN-GA <sup>-</sup> CAFHH <sup>-</sup> ELKQAI---CRC <sup>-</sup> FTGYTG <sup>-</sup> QRC <sup>-</sup> EHL <sup>-</sup> TLT <sup>-</sup> SYA....	26
Trans. mouse genomic locus	...	VALKFSHP <sup>-</sup> CL <sup>-</sup> EDHNSY <sup>-</sup> CIN-GA <sup>-</sup> CAFHH <sup>-</sup> ELKQAI---CR*	83, 138
Trans. mouse genomic locus	...	I <sup>-</sup> ALKFSHL <sup>-</sup> CL <sup>-</sup> EDHNSY <sup>-</sup> CIN-GA <sup>-</sup> CAFHH <sup>-</sup> ELEKAI---CR*	84, 139

FIGURE 4B

# Replacement Sheet

i) TGF alpha Sequence ID #  
 EGF DOMAIN NUMBER -----SEQUENCE-----  
 1. EGF\_47\_82\* CPDSHTQFCF-HGT-CRFLVQEDKPACVCHSG--YVGAR--C 72

ii) EPIDERMAL GROWTH FACTOR Sequence ID #  
 EGF DOMAIN NUMBER -----SEQUENCE-----  
 1. EGF\_318\_354 CKLR-KGNCS--STVCGQDL--QSHLCMCAEGYALSRDRKYC 27  
 2. EGF\_360\_395 CAFW-NHGC---TLGCKNTP--GSYYCTCPVGFVLLPDGKRC 28  
 3. EGF\_401\_436 CPRN-VSEC---SHDCVLTS--EGPLCFCPEGSVLERDGKTC 29  
 4. EGF\_439\_476 CSSPDNGGCS--QLCVPLSP--VSWECDCFPGYDLQLDEKSC 30  
 5. EGF\_745\_780 CLYQ-NGGC---EHICKKRL--GTAWCSCREGFMKASDGKTC 31  
 6. EGF\_835\_868 CAPV---GCS-MYARCISEG--EDATCQCLKG--FAGDGKLC 32  
 7. EGF\_874\_910 CEMG-VPVCPPASSKCINTE--GGYVCRCSEG--YQGDGIHC 33  
 8. EGF\_916\_951 CQLG-VHSCGENAS-CTNTE--GGYTMCAGR--LSEPGLIC 34  
 9. EGF\_976\_1012\* CPLSHDGYCL-HDGVCMYIEALDKYACNCVVG--YIGER--C 35

FIGURE 5B

## iii) Notchl

Sequence ID #

EGF DOMAIN NUMBER	SEQUENCE	
1. EGF_24_57	CSQ-----PGETCLNGGKCEAANGTE-----ACVCG-GAFVVGPRC	36
2. EGF_63_98	CLS-----TPCKNAGTCH-VVDRRGVADYACSCA-LGFSGPLC	37
3. EGF_106_138	CLT-----NPCRNGGTCD-LLTLT---EYKCRCP-PGWSGKSC	38
4. EGF_144_175	CAS-----NPCANGGQC---LP-FEASYICHCP-PSFHGPTC	39
5. EGF_182_215	CGQ-----KPRLCRHGGTCHNEVGSY-----RCVCR-ATHTGPNC	40
6. EGF_222_254	CSPSP-----CQNGGTCRPTGDV-----THECACL-PGFTGQNC	41
7. EGF_261_292	CPGNN-----CKNGGACV-DGVN---TYNCPCP-PEWTGQYC	42
8. EGF_299_332	CQL-----MPNACQNGGTCHNTHGGY-----NCVCV-NGWTGEDC	43
9. EGF_339_370	CAS-----AACFHGATCH-DRVA---SFYCECP-HGRTGLLC	44
10. EGF_376_409	CIS-----NPCNEGSNCD-TNPV---NGKAICTCP-SGYTGPA	45
11. EGF_416_449	CSL-----GANPCEHAGKCINTLGSF-----ECQCL-QGYTGPRC	46
12. EGF_456_487	CVS-----NPCQNDATCL-DQIG---EFQCMCM-PGYEGVHC	47
13. EGF_494_525	CAS-----SPCLHNGRCL-DKIN---EFQCECP-TGFTGHLC	48
14. EGF_532_563	CAS-----TPCKNGAKCL-DGPN---TYTCVCT-EGYTGTHC	49
15. EGF_570_600	CDPDP-----CHYG-SCK-DGVA---TFTCLCR-PGYTGHH	50
16. EGF_607_638	CSS-----QPCRLRGTCQ-DPDN---AYLCFCL-KGTTGPNC	51
17. EGF_645_675	CAS-----SPC-DSGTCL-DKID---GYECACE-PGYTGSMC	52
18. EGF_682_713	CAG-----NPCHNGGTC---ED-GINGFTCRCP-EGYHDPTC	53
19. EGF_720_750	CNS-----NPCVHGACRD---SLN---GYKDCD-PGWSGTNC	54
20. EGF_757_788	CES-----NPCVNGGTCK-D-MTS---GIVCTCR-EGFSGPNC	55
21. EGF_795_826	CAS-----NPCLNKGTC---IDD---VAGYKCNCL-LPYTGATC	56
22. EGF_833_867	CAP-----SPCRNGGECR-QSED-YES-FSCVCPTAGAKQTC	57
23. EGF_874_905	CVL-----SPCRHGASCQ-NTHG---XYRCHCQ-AGYSGRNC	58
24. EGF_912_943	CRPNP-----CHNGGSCT-DGIN---TAFCDCL-PGFRGTFC	59
25. EGF_950_981	CAS--DP-----CRNGANCT-DCVD---SYTCTCP-AGFSGIHC	60
26. EGF_988_1019	CTESS-----CFNGGTCV-DGIN---SFTCLCP-PGFTGSYC	61
27. EGF_1026_1057	CDS-----RPCLLGGTCQ-DG---RGLHRCTCP-QGYTGPN	62
28. EGF_1064_1095	CDS-----SPCKNGGKCW-QT---HTQYRCECP-SGWTGLYC	63
29. EGF_1102_1143	CEVAAQRQGVVDVARLCQHGGLCV-DAGN---THHCRCQ-AGYTG	64
30. EGF_1150_1181	CSP-----SPCQNGATCT-DYLG---GYSCKCV-AGYHGVNC	65
31. EGF_1188_1219	CLS-----HPCQNGGTCL-DLPN---TYKCSCP-RGTQGVHC	66
32. EGF_1226_1265	CNPPVDPVSR--PKCFNNGTCV-DQVG---GYSCTCP-PGFVG	67
33. EGF_1272_1305	CLS-----NPCDARGTON-CVQR---VNDFHCECR-AGHTG	68
34. EGF_1312_1346	CKG-----KPCCKNGGTCA-VASN-TARGFICKCP-AGFEGATC	69
35. EGF_1353_1384	C-----GSLRCLNGGTCTISGPRSP-----TCLCL-GPFTG	70
36. EGF_1392_1426	CL-----GGNPCYNQGTCEPTSESPF---YRCLCP-AKFENGLLC	71

FIGURE 5C

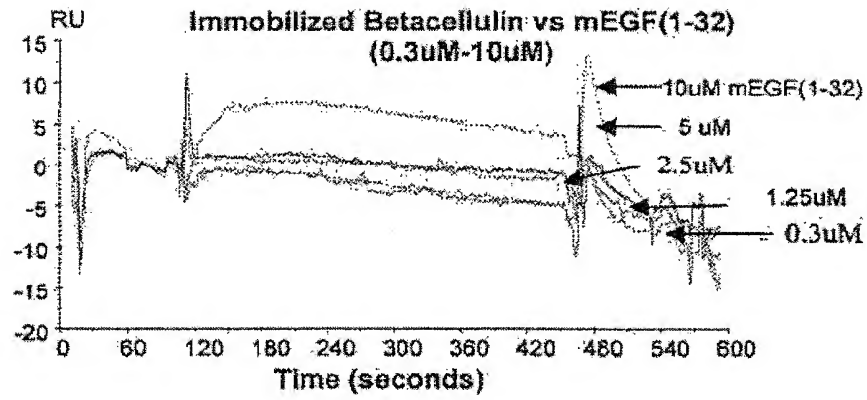


FIGURE 6A

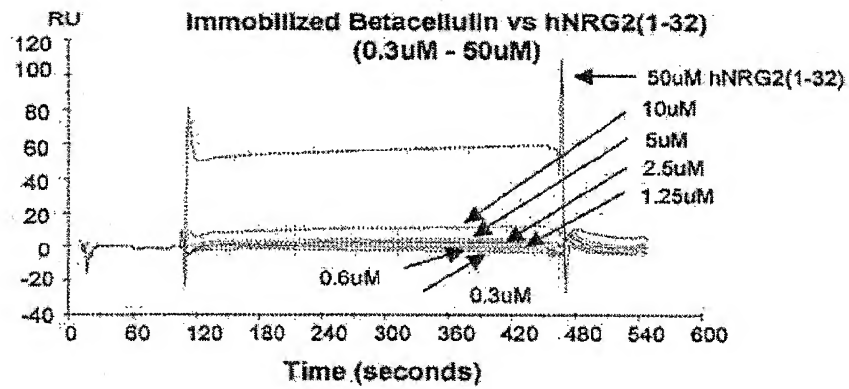


FIGURE 6B